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Reflectance properties and physiological responses of *Salicornia virginica* to heavy metal and petroleum contamination

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Spectral characteristics of S. virginica were significantly correlated with symptoms of cadmium and lightweight petroleum contamination, and had a distinct signature for both types of contaminants.

Abstract

Wetland ecosystems of California are located in highly populated areas and subject to high levels of contamination. Monitoring of wetlands to assess degrees of pollution damage requires periodic retrieval of information over large areas, which can be effectively accomplished by rapidly evolving remote sensing technologies. The biophysical principles of remote sensing of vegetation under stress need to be understood in order to correctly interpret the information obtained at the scale of canopies.

To determine the potential to remotely characterize and monitor pollution, plants of *Salicornia virginica*, a major component of wetland communities in California, were treated with two metals and two crude oil types to study their sensitivity to pollutants and how this impacted their reflectance characteristics. Several growth and physiological parameters, as well as shoot reflectance were measured and correlated with symptoms and contamination levels.

Significant differences between treatments were found in at least some of the measured parameters in all pollutants. Reflectance was sensitive to early stress levels only for cadmium and the lightweight petroleum. Pollutants that differ in their way of action also had different plant reflectance signatures. The high degree of correlation between reflectance features and stress indicators highlights the potential of using remote sensing to assess the type and degree of pollution damage.

Keywords: Cadmium; Oil; Remote sensing; Vanadium; Wetlands

1. Introduction

California was once rich in highly productive wetlands, which served as habitats for numerous species. The structure and ecological functioning of many of these habitats have been lost due to hydrologic alterations such as construction of dikes, and levees for water diversion toward agricultural or urban land uses (Macdonald, 1977). Industrial development brought additional sources of wetland disturbance. Coastal marshes are particularly sensitive to industrial pollutants, which are transported by tidal flooding that can be deposited into the sediment. Salt marshes are also particularly vulnerable to chemical pollution because they are subject to little turbulent mixing and their substrates are fragile (Venosa et al., 2002). Among the most important industrial pollutants are crude petroleum, petroleum byproducts and heavy metals. Both heavy metals and petroleum oils are known to cause stress in estuarine vegetation (Mendelssohn et al., 2001) but the nature of their effects and how to diagnose these

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effects in coastal salt-marsh vegetation are not well established.

Monitoring the remaining wetlands is needed to detect ecosystem stressors and to predict adverse habitat changes. Because of the difficulty in accessing wetlands on the ground and cost of field-based monitoring, methods that can provide quick assessments are essential to detect early changes that may affect the ecology of the marsh. Remote sensing is a valid alternative to traditional ground-based methods to detect plant stress, especially since the emergence of high spectral resolution imaging sensors. Remote sensing instruments measure radiance reflected from the leaf and canopy surfaces. The spectral differences in these reflectances are derived from leaf optical properties related to the physiological and biophysical status of the plants. Leaf optical properties are a function of leaf cellular structure, water content and biochemical composition (cellulose, lignin, starch, proteins, sugars, etc) and pigment concentrations (Asner, 2004; Ustin et al., 1999, 2004; Wessman, 1990; Woolley, 1971). Alterations in plant biochemistry and cellular composition imposed by environmental stressors produce changes in the reflectance characteristics that can be detected using remote sensors. In this paper, we assess the potential for using remote sensing data to detect and monitor environmental stressors, using a series of contamination experiments.

Tidal marsh ecosystems of California are dominated by a few species, which occupy well-distinguished growth zones that are determined predominately by tolerance to salinity levels and timing and duration of tidal inundations (Pennings and Callaway, 1992). San Francisco Bay salt-marshes are dominated by *Salicornia virginica*, a succulent halophytic chenopod species that occupies the higher reaches of the marsh environment. This species is highly salt tolerant (Zedler et al., 2003), but its tolerance to petroleum and heavy metals has not been studied.

Among the common coastal pollutants, cadmium (Cd) is the non-essential heavy metal that has attracted the most attention due to its toxicity to humans (McLaughlin and Singh, 1999). Main sources of Cd contamination are fertilizers, solid waste, metal industry uses, and mining (Alloway and Steinnes, 1999). Contamination with vanadium (V) also can be high in coastal sites where fuel combustion occurs (Bu-Olayan and Al-Yakoob, 1998) and it has been used as an indicator of release of other trace metals (Soldi et al., 1996). Salt marsh ecosystems are at high risk from crude oil contamination because of their proximity to oil production, refining and trans-shipment. Oil type and dosage are two of the most important variables to be taken into account when judging the potential damage from oil contamination in plants and the environment (Lin et al., 2002; Pezeshki et al., 2000).

In this paper, we assess the effect of two heavy metals: Cd and V, and two crude oil types: 'Escravos' and 'Alba', on the growth and physiology of *Salicornia*, with two primary objectives: (1) To evaluate the possibility of detecting a spectrally specific response in vegetation induced by a particular metal or crude petroleum extract; (2) To evaluate the magnitude of *Salicornia* responses to different concentrations of the pollutants.

2. Materials and methods

2.1. Plant material

Seeds of *S. virginica* were collected at the brackish Petaluma marsh, about 9 km north of San Pablo Bay, California. Seeds were germinated in a greenhouse at University of California, Davis, in 10 cm wide pots filled with horticultural sand. Pots were placed in a tray to partially submerge them in aerated 0.5× Hoagland's Solution. Supplemental lighting was provided for 12-h days for several weeks until plants grew to an approximate height of 10 cm. They were then individually transplanted into perlite medium in their final containers, plastic 25 cm long by 6.5 cm wide pots (commonly used for planting tree seedlings). About a week was given for acclimation and plants showing visible stress symptoms (e.g., wilting, chlorosis) were eliminated from the experiment.

2.2. Pollutant exposure experiments

Experiments were conducted outdoors at U.C. Davis in full sun between May and October 2002. Experiments included a set of control plants and two to four treatments at varying levels of metal or petroleum concentration. Each metal or petroleum treatment was applied to plants growing in separate metal tubs (80 L each) and all tubs of a given pollutant were treated simultaneously. Metal tubs were lined with a 6-mm grade plastic sheeting. Twenty individual plant pots were randomly chosen, arranged in a tube rack, and placed in each treatment tub. Plant racks were randomly assigned to tubs. Tubs were filled with an aerated 100% strength Hoagland's nutrient solution salinized with 8000 ppm NaCl. A week was given for acclimation before the experiments were started.

Since part of the objectives of this experiment was to determine the sensitivity of *Salicornia* to pollutants, and because lethal and harmful levels of contamination were unknown, a dynamic approach was adopted to determine the dosage. A low dosage of pollutants was initially applied and gradually increased until stress symptoms appeared. Experiments started by randomly choosing a control tub and the rest of the tubs were given the lowest level of the treatment following a pre-established increment (Table 1 and Section 3). Out of the treated tubs, a second tub was randomly chosen to remain at the lowest dose, and the dose in the remaining

Table 1
Concentrations of final pollutant levels and chemical analyses at the end of experiments

Pollutant	Final treatment levels	Tub water chemical analysis	Shoot chemical analysis	Root chemical analysis
Cadmium (ppm)	0, 12, 24, 36, 48	0.2, 12.0, 23.1, 32.9, 45.8	17, 86, 100, 180, 250	162, –, –, –, 627
Vanadium (ppm)	0, 36, 45, 54	0.1, 35.1, 44.0, 54.7	14, 41, 68, 149	13, 1953, 3083, 3128
Petroleum 'Escravos' (%)	0, 0.7, 1.4	–	–	–
Petroleum 'Alba' (%)	0, 7.7, 9.1	–	–	–

ppm = parts per million, % = percent of soil weight.

tubs was increased to the next treatment level. This procedure continued until all tubs were treated. If after the highest dose symptoms were not apparent, then an additional increment was applied to all treatment tubs.

To expose the plants to Cd and V, cadmium chloride and vanadium sulfate, respectively, were added by weight to the salinized solution. The volume of solution in the treatment tubs was carefully monitored and kept at a constant level for the duration of the experiment to avoid changes in concentration due to water loss from evapotranspiration. At the end of each metal experiment, the solution in each tub was analyzed to determine the final level of pollutants.

The petroleum exposure experiments were done with two types of crude oil, 'Escravos' petroleum (EP), a light molecular weight petroleum (specific gravity = 0.85); and 'Alba' (AP), a heavier weight petroleum (specific gravity = 0.94). Due to the low solubility of petroleum in water, and to provide uniformity in the treatments, 12-cc syringes were used to inject the petroleum into each individual plant container, so the treatment was mostly confined to the rooting zone of each plant. To simulate tidal dynamics and to allow for the oil to penetrate the soil and contact the entire root system, water level was lowered daily by siphoning the solution out of each tub, and then carefully poured back from the top.

2.3. Plant growth and physiological measurements

Chlorophyll fluorescence (ChFl) was measured every three days from the beginning of the experiments to monitor the onset of stress and determine the effect of the metal salts and petroleum. ChFl is considered a good estimator of photosynthesis quantum efficiency (Belkhodja, 1994; Schuerger et al., 2003). Fluorescence was measured pre-dawn using the Walz PAM 2000 (Heinz Walz GmbH, Effeltrich, Germany). The fluorescence parameters variable fluorescence (Fv) and maximum fluorescence (F_m) were recorded and their ratio was calculated and averaged per tub. Fv is the difference between minimum (in absence of light) and maximum (light-induced) fluorescence. Fv/Fm measures the proportion of maximum possible fluorescence used for photosynthesis, which tends to decrease with stress. Five shoots per treatment (tub) were randomly chosen for these measurements. Shoots showing extreme symptoms of stress relative to the appearance of most shoots in the tub were not measured. Although this protocol biased samples toward the least symptomatic shoots, it avoided including plants that were under stress for reasons other than the effect of pollutants. Prior to application of a dose of heavy metal or petroleum, an average base line ChFl measurement was established for each treatment level.

Photosynthetic activity was measured at the termination of each experiment, using a field-portable infrared gas analyzer (LI-6400, LI-COR, Lincoln, NE). Photosynthesis was measured between 11:30 and 13:30 am standard time. Measurements were made on 10 randomly chosen plants on the third branch of the main stem, starting from the plant's apex, on a location 5 cm from the branch node. Measurements were made along a standard 2 cm length of stem. Cylindrical surface area was calculated based on mean stem diameters measured by electronic caliper to 0.1 mm, to compute gas exchange rates. The LiCOR 6400 was equipped with an LED light source, which matched incident and measured irradiances and a CO_2 injection system that was adjusted to maintain constant CO_2 ambient exposure levels during the measurements.

Plant height and stem diameter were measured on all the 20 plants of each treatment level. Plant height was measured from the level of the perlite surface to the apex of the largest shoot in the individual pots. Stem diameters were measured to the nearest 0.1 mm, using an electronic caliper, at the location where gas exchange was measured. Plant water content per treatment level was calculated by subtracting dry weight from fresh weight (measured to 0.001 g) of 10 plants per tub (chosen randomly), and then averaged.

Photosynthetic tissue samples were extracted from the location where gas exchange was measured. Stem segments were placed in glass vials containing 2 ml of *N*,*N*-dimethyl formamide to extract Chl and placed in a refrigerator until measured. Tissue suspensions were filtered and measured with a diode-array spectrophotometer (model 8456A, Hewlett Packard) and pigment concentration was calculated according to the procedures of Wellburn (1994).

Averages and standard errors were calculated for all the data. Parameters were examined for significant effects of metal salts and petroleum using analysis of variance (ANOVA). Post hoc test analyses were conducted on

ANOVA to determine significant differences using a least squares means multiple comparison procedure.

2.4. Leaf level reflectance

At each treatment level, a minimum of 10 plants was randomly selected to obtain reflectance spectra. A stem segment from each plant was identified following the protocol used for the gas exchange measurements. Plants showing signs of advanced senescence (relatively to most plants in the tub), or extensive damage, were not measured. Three to five replicates of reflectance were collected for each segment using either an ASD FR (Analytical Spectral Devices, Boulder, CO) or a GER 2600 (Geophysical & Environmental Research Corporation, Buffalo, New York) spectrometer (depending on availability) attached to a LiCOR 1800 Integrating Sphere (Lincoln, NE). The full-width-half-maximum spectral response of the ASD is 3 nm from 350 to 1000 nm region and 10 nm from 1000 to 2500 nm. The LiCOR modified the light source by removing the heat filter, allowing light at longer infrared wavelengths to be emitted from the laser, which permitted spectral measurements at longer wavelengths. A black felt filter was used in the aperture of the sphere to prevent light from passing around the edges of the shoot. The filter had a slot cut into it which sealed around the stem segment for measurements.

Individual reflectance spectra were averaged by treatment. Spectra were filtered for bad data and outliers based on visual inspection of the individual spectra. Wavelengths shorter than 450 nm and longer than 1700 nm were not analyzed due to excessive noise. Spectra were normalized at the 500 nm band using the average spectrum of the control treatment as a reference. This removed some variability due to albedo differences that were independent of wavelength specific responses while retaining the spectral variability between samples. Differences between treatment levels and control were computed for the entire spectrum. Wavelengths that are biophysically relevant and wavelengths at which variation between treated plants and controls were higher were selected for ANOVA comparisons to test for statistical significance of differences.

2.5. Chemical analysis

Water samples were collected from treatment tubs at the end of the experiments and analyzed at the DANR Analytical Lab (University of California, Davis) for heavy metal concentrations. Dry plant samples used to estimate water content were ground with a Wiley Mill to obtain a uniform particle size of 40 μm and sent to the DANR Laboratory for metal analysis. No test was available for measuring petroleum concentration in plants.

3. Results

3.1. Cadmium

Cd treatment levels were administered at 12 ppm increments. After 10 days the highest level was achieved, and three days later first visual symptoms appeared. The most salient symptom was shoot and leaf chlorosis and to some extent, thinner canopies (Fig. 1). Chemical analyses of water and plant material at the end of the experiment showed a progression of contamination levels consistent with the intended doses. Low (L) and the highest (H) treatment levels showed the most extreme signs of stress (Fig. 1), despite the progression of increasing concentrations shown in the chemical analyses (Table 1).

Although the control (C) had always the highest ChFl values, Fv/Fm means at the end of the experiment were not significantly different among treatments. However, in earlier dates ChFl was higher for the C and medium-high (MH) treatments (Fig. 2 and Table 2), which was consistent with visual symptoms (Fig. 1). Pigment concentration patterns among treatments (Table 2) closely matched the visual chlorosis levels and the fluorescence values in the middle of the experiment. Although with not so well defined mean differences, gas exchange followed a pattern similar to pigment concentrations (Table 2).

Leaf reflectance for the visible and near-infrared (NIR) wavelengths followed the observed trends in chlorosis, with C being the least affected and MH, and L, ML, and H being increasingly more affected (Figs. 3a). ANOVA showed significant treatment differences with respect to the C reflectance at the wavelengths tested (Fig. 3b). Visible reflectances showed a tight, inverse linear relationship with pigment concentration (Fig. 4a), and with photosynthesis (Fig. 4b). NIR reflectance was to some extent proportional to shoot dry weight (Fig. 4c). Wavelengths longer than 1000 nm, however, followed a progression that closely matched the treatment levels (Fig. 4d).

3.2. Vanadium

V contamination levels were administered at 9 ppm increments. After reaching a maximum of 27 ppm, all contamination levels were increased three more times (resulting in 36, 45 and 54 ppm, Table 1) until the appearance of symptoms, 19 days from the beginning of the experiment. The chemical analyses of the treatment solutions at the end of the experiment approximately matched the intended concentrations (Table 1). The most evident symptom of stress was shoot mortality, some stunting and a moderate chlorosis (Fig. 1), in a progression that approximated treatments and plant concentration analyses (Table 1).

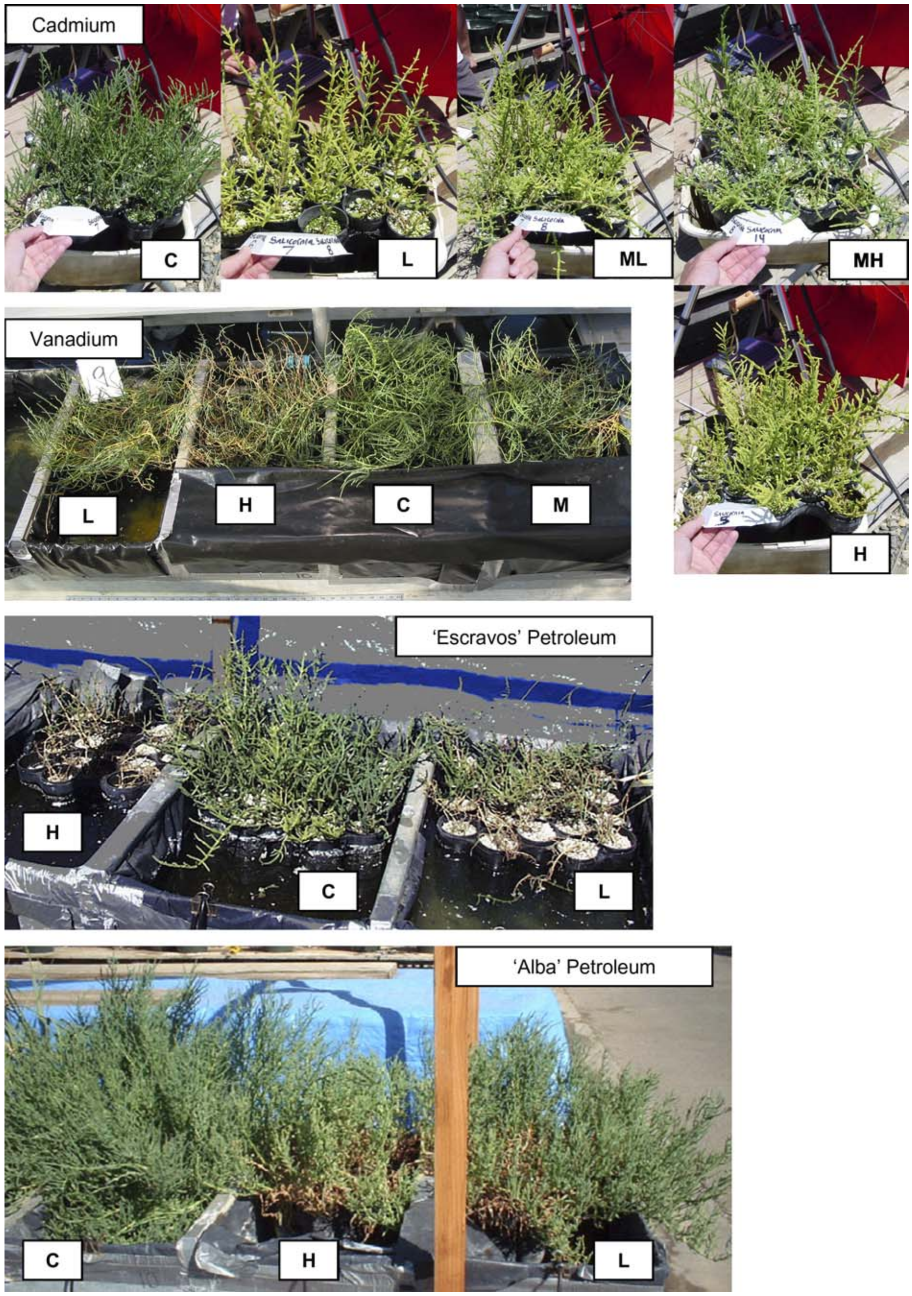

Fig. 1. Visual symptoms of *Salicornia* according to treatment levels of the four pollutants tested. C = control, L = low, M = medium, H = high, ML = medium-low, MH = medium-high.

ChFl did not show any significant trend, and values fluctuated throughout the experiment. Plant measurement results are presented in Table 3. Leaf reflectance did not closely follow treatment levels (Fig. 5a) and reflectance of treatments was not statistically different from C (Fig. 5b).

3.3. 'Escravos' petroleum

EP contamination levels were administered at 1 ml (0.7% of soil weight) increments. Three days after the second level was reached (Table 1) (10 days after the beginning of the experiment) visual symptoms appeared. Stunting and shoot mortality progressed as treatment levels increased. All treated plants showed liquid brown secretions at some stem nodes, which eventually led to yellowing at the base of the internodes.

ChFl differences among treatments were not significant ($p = 0.05$) but for the last two weeks of the experiment the H level was consistently the lowest. Control treatment was significantly higher in all the morphological parameters (Table 4), there were no

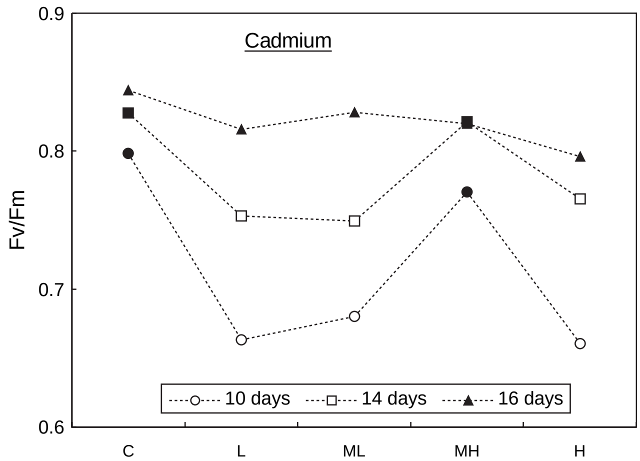

Fig. 2. Evolution of Chl fluorescence of the Cd experiment. Days are counted since the beginning of the experiment. Markers with different colors indicate means that are statistically different ($p = 0.05$) within a given date. C = control, L = low, ML = medium-low, MH = medium-high, H = high.

differences in the pigment concentrations among treatments, but photosynthesis activity was significantly higher in C than in all treatments.

The H treatment had a distinct pattern of leaf reflectance compared to the C treatment. Across most of the spectrum, reflectance was lower in the former (Fig. 6a). Reflectance was higher in the H only in regions of deep absorption features, such as at 670 nm. C and L were never significantly different, but significantly different from H in regions of greatest spectral difference (Fig. 6b).

3.4. *'Alba' petroleum*

AP contamination levels were administered at 1 ml (0.7% of soil weight) increments. Five days after the start of the experiment the L and H levels were reached. One increment was added to both levels every three days. Since after 23 days from the start of the experiment no effects could be detected, the difference between pollutant levels was increased to two increments. Symptoms became apparent after 9 days (32 days total). A relatively slight chlorosis, a decrease in stem height and some wilting and mortality of stems in the basal portion of plants characterized the signs of stress. Some contaminated plants showed brown secretion as in EP at some stem nodes, but these did not necessarily result in wilting.

ChFl differences were non-significant and values fluctuated throughout the experiment with no definite trend. Plant measurement results are presented in Table 5. Photosynthesis activity of C was significantly higher than the H treatment.

Mean leaf reflectance of L appeared different from C and H in the visible range (Fig. 7a) although differences were not significant at this portion (Fig. 7b). Reflectance followed the expected pattern of treatment between 1000 nm and 1300 nm and differences between H and C were significant at 1050 nm.

4. Discussion

Leaf reflectance of contaminated plants showed that in at least some cases there were sufficient changes in spectra of stressed plants to be remotely detected. In general, significant differences in spectral responses were less frequent than significant differences in morphological, physical and physiological parameters. In some cases, a bias towards 'normal' reflectance could have been introduced by discarding plants showing extreme symptoms. However, this is not the case in experiments in which plants showed uniform symptoms within treatments, as in Cd or AP (Fig. 1). Variations in response of these parameters across chemicals suggest that the mechanisms and nature of stress varies between pollutants. The effects on reflectance of different pollutants seem to correspond with the general visual appearance of treated plants (Fig. 1), which show more evident symptoms in Cd and EP experiments, than in V and AP. This is not surprising since visual appearance integrates many stress symptoms and responses such as growth, chlorosis, wilting and mortality, resulting in an overall indicator of plant health. Moreover, visual appearance was more reliable as a stress predictor than leaf ChFl, which except for the Cd experiment during

Table 2
Effect of Cd concentration on plant biometric and physiological parameters

Parameter	Treatment					Significance
	C	L	ML	MH	H	
Fv/Fm (last date)	0.84	0.82	0.83	0.82	0.80	ns
Height (mm)	243.1	220.8	280.8	237.9	225.7	ns
Stem thickness (mm)	2.53	2.27	2.47	2.54	2.61	ns
Shoot dry weight (g)	2.44 a	1.07 b	1.76 ab	1.57 ab	1.81 ab	0.01
Shoot water content (%)	90.79 a	89.91 a	88.23 b	90.75 a	89.87 a	0.05
Total pigment ($\mu g\,cm^{-2}$)	28.93 a	6.26 b	10.43 c	16.75 d	8.10 c	0.01
CO_2 assimilation ($\mu mol\ CO_2\ min^{-1}\,cm^{-1}$)	4.97 a	0.46 bc	0.68 bcd	3.03 acd	1.97 bcd	0.01

Treatment levels: C = control, L = low, ML = medium-low, MH = medium-high, H = high. Significance: ns = non-significant at the 0.05 level. Means with different letters are significantly different at the 0.05 level.

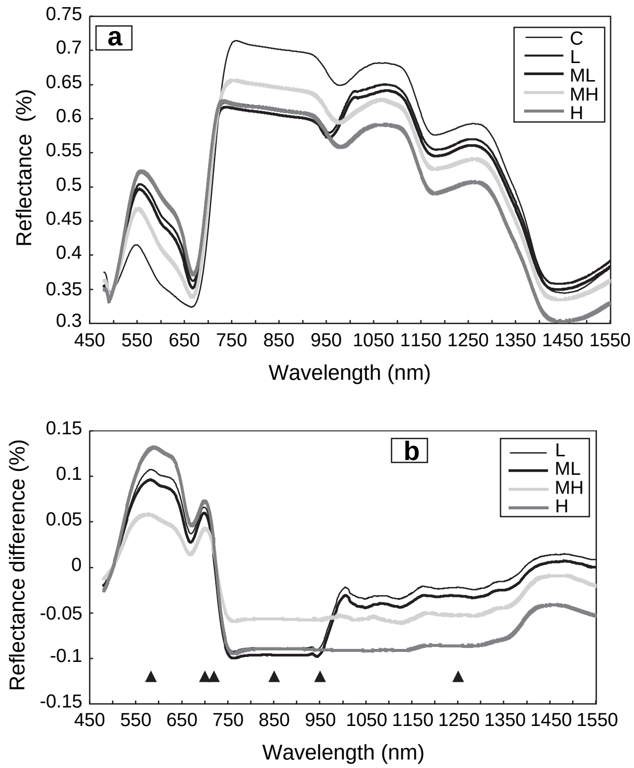

Fig. 3. (a) Reflectance spectra of Cadmium experiments, (b) differences between treatment and control spectra. Triangles indicate wavelengths at which differences were tested for significance. Black triangles indicate significant differences at the 0.05 level. C = control, L = low, ML = medium-low, MH = medium-high and H = high.

certain periods (Fig. 2) did not show much sensitivity to stress. Similar results regarding the relatively lack of sensitivity of the Fv/Fm ratio are reported by Mendelssohn et al. (2001), although it has been found to be positively correlated with Chl content elsewhere (Subhash et al., 1999).

That V and AP had less dramatic toxic effects than Cd and EP is also demonstrated by the fact that higher contamination concentrations and a longer duration of exposure were necessary to produce symptomatic responses. Since only one heavy metal experiment and one petroleum experiment showed significant effects on reflectance, it is difficult to make valid comparisons of the generalized effects of heavy metals versus petroleum. The only significant difference in reflectance found in the two petroleum experiments, at approximately 1050 nm, shows the opposite relationship between C and H treatments (Figs. 6b and 7b). Carter (1993) when comparing the response in reflectance to several stress factors found that infrared generally did not change or changed inconsistently among these factors.

Cd reflectance shows significant differences across treatments in the visible range. Starting from C, which has the lowest reflectance values, the progression towards higher values follows very closely the order of symptom severity (mainly chlorosis) and pigment concentration (Fig 4a and Table 2). Carter (1993) found a significant increase in reflectance around the 550 nm (green) and the 710 nm (red) due to various stresses, which is exactly where we found the highest increases (Fig. 3b). These responses can be linked to Chl concentration reductions (Carter and Knapp, 2001). Chla has relatively low absorptivity at these wavelengths and therefore, any decrease in pigments would result in higher reflectance (Carter, 1993). Interestingly, symptoms developed in a progression that does not reflect the contamination levels administered. The results of the water and plant Cd chemical analyses at the end of the experiment eliminate the possibility of an error in chemical administration or a mislabeling of treatment tubs.

Reflectance differences in the near infrared (NIR) portion followed a similar progression as the symptom expression, but in contrast to visible wavelengths, towards a reduction in reflectance with stress (Fig. 4c). NIR reflectance is strongly determined by the structural characteristics of leaf parenchyma, fractions of air spaces and air–water interfaces (Jacquemoud et al., 1996; Ustin et al., 1999). A reduction in intercellular spaces produces less light scattering and less reflectance. Thus, water stress influences reflectance at the NIR region because of changes in mesophyl structure, and at the short wave infrared (SWIR) region because of reduction of water content (Bowman, 1989). Some parameters such as stem thickness and shoot water content that could have been consistent with the reflectance changes observed, either were not significantly different or did not agree with the patterns of reduction in reflectance. As expected, shoot dry weight showed some degree of correlation with reflectance at 850 nm (NIR) (Fig. 4c), however, better correlations have been found when dry weight was expressed as unit of leaf area (Jacquemoud et al., 1996). Significant differences were found at 1240 nm and the same pattern can be observed through 1550 nm, the SWIR region of the spectrum. In these portions, there is a progression that more closely follows the contamination levels rather than the symptoms observed (Fig 4d). This tight relationship suggests that the pollutant produced a stress in treated plants somewhat independently from the changes in pigments (and chlorosis) evident in the visual and NIR portions. Ceccato et al. (2001) showed that leaf structural characteristics have more influence in reflectance at 820 nm than at higher wavelengths, and that water content has no effect at 820 nm but it is highly determinant of reflectance at longer wavelengths.

Cd has been found to inactivate plant hormones such as cytokinin (Veselov et al., 2003), which produces reduction in photosynthesis and cell membrane damage (Prasad, 1995). It also reduces electron transport in the chloroplast photosystems (Cheng et al., 2002). V inhibits various enzyme systems, affecting plant growth and

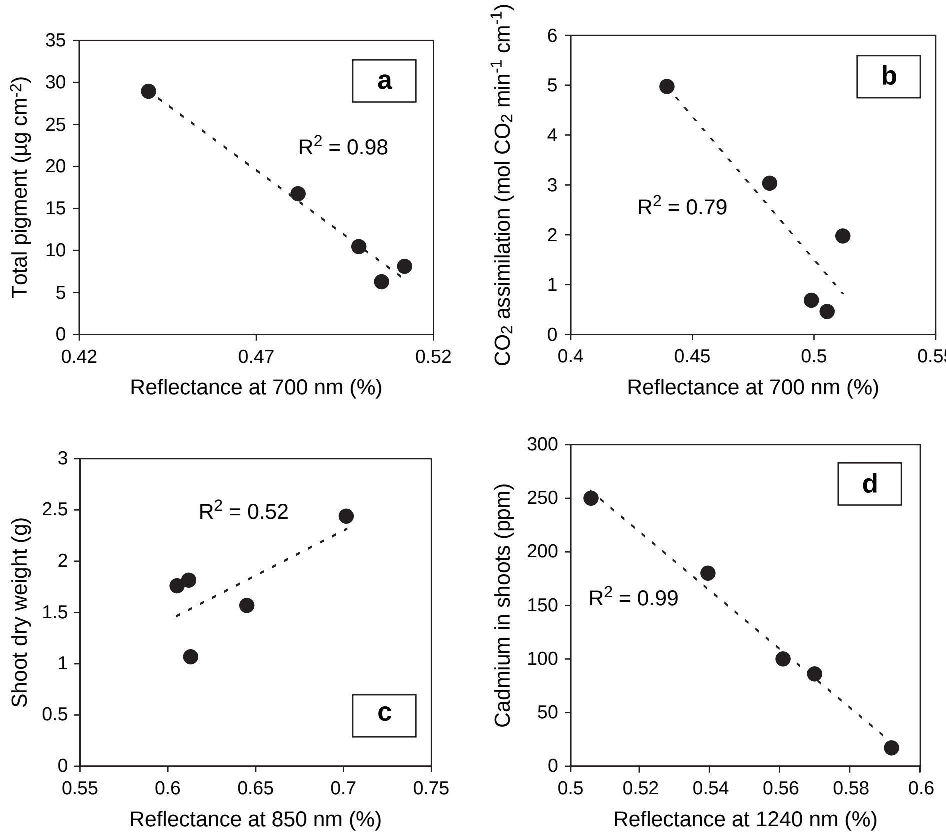

Fig. 4. Relationship between reflectance and plant measured parameters in the Cd experiment. (a) 700 nm (visible region) and pigment content; (b) 700 nm (visible region) and photosynthesis rate; (c) 850 nm (NIR region) and shoot dry weight; and (d) 1240 nm (SWIR region) and Cd concentration in shoots.

producing chlorosis (Peterson and Girling, 1981). This is consistent with our results (Table 3), although a decrease in pigment contents did not result in significant reductions of photosynthesis. Even though metals tend to accumulate and be retained in the roots, Cd is relatively mobile and tends to be more evenly distributed in the plant (Williams et al., 1994). This explains the differences of one order of magnitude between Cd and V observed in the root chemical analyses (Table 1), and is probably related to the higher sensitivity of *Salicornia* to Cd.

EP also produced significant changes in leaf reflectance. The H treatment level showed a generalized decrease in reflectance, except for small regions around 490 nm (blue), 670 nm (red) and 1530 nm (SWIR), where H had higher reflectance than both C and L (Fig. 6). The lack of significant changes in pigment concentration (Table 4) and the relatively little difference in apparent plant chlorosis (Fig. 1) suggest a qualitatively different response to EP contamination compared to Cd and V. Plants contaminated with EP experienced a relatively sudden mortality without going

Table 3
Effect of V concentration on plant biometric and physiological parameters

Parameter	Treatment				Significance
	C	L	M	H	
Fv/Fm (last date)	0.82	0.82	0.83	0.84	ns
Height (mm)	413.5 a	304.3 b	348.6 b	343.8 b	0.01
Stem thickness (mm)	2.51 a	2.20 b	2.01 b	2.04 b	0.01
Shoot dry weight (g)	6.87	3.66	3.74	3.03	ns
Shoot water content (%)	88.23 a	78.93 b	78.59 b	75.06 b	0.01
Total pigment ($\mu g\ cm^{-2}$)	5.68 a	–	10.71 b	15.36 c	0.01
CO_2 assimilation ($\mu mol\ CO_2\ min^{-1}\ cm^{-1}$)	4.75 a	4.50 a	2.39 b	3.69 a	0.01

Treatment levels: C = control, L = low, M = medium, H = high. Significance: ns = non-significant at the 0.05 level. Means with different letters are significantly different at the 0.05 level.

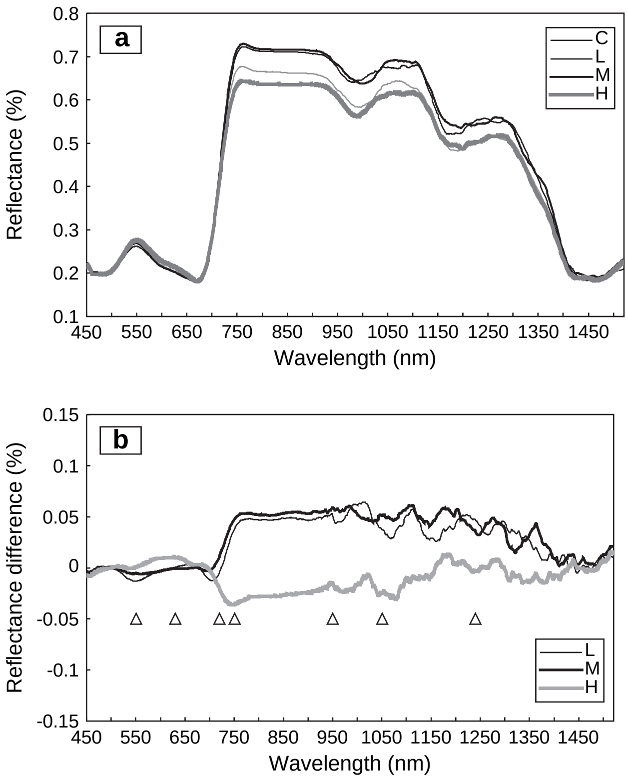

Fig. 5. (a) Reflectance spectra of Vanadium experiments, (b) differences between treatment and control spectra. Triangles indicate wavelengths at which differences were tested for significance. White triangles indicate non-significant differences at the 0.05 level. C = control, L = low, M = medium, and H = high.

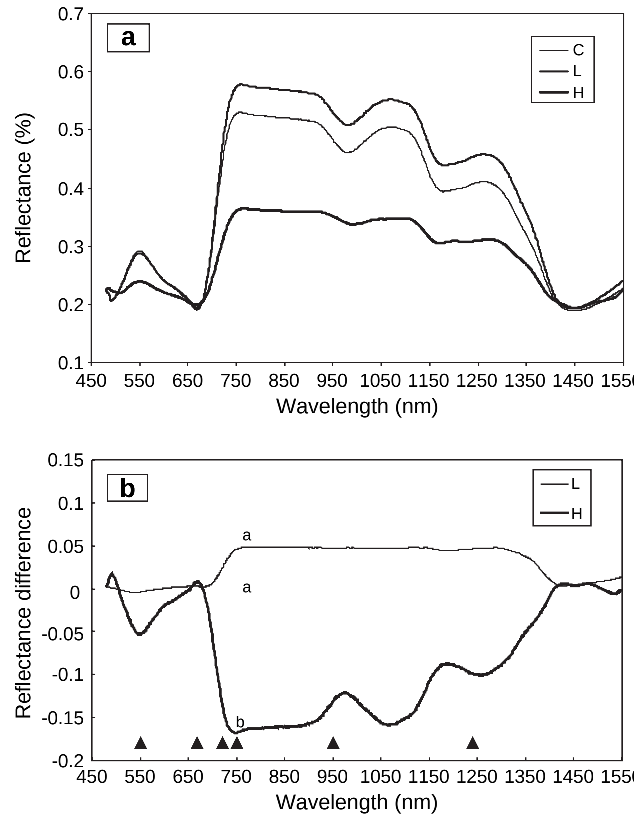

Fig. 6. (a) Reflectance spectra of 'Escravos' petroleum experiments, (b) differences between treatment and control spectra. Triangles indicate wavelengths at which differences were tested for significance. Black triangles indicate significant differences at the 0.05 level. Spectra with different letters are significantly different (0.05 level) at the four NIR wavelengths tested. C = control, L = low, H = high.

through an extended period of yellowing. The main effect of EP may not be directly on the pigment system but at a structural or chemical level with no evident visual symptoms. Jensen (2000) in describing different stages of decline in sweetgum and blackjack oak leaves, indicates that while yellow leaves show increases in reflectance similar to the ones observed in Cd, spectra of brown leaves are flattened relative to the typical plant spectral curves with reduced reflectance in the green and NIR, and an increase in the red region. The gradual reflectance response to petroleum contamination can also be illustrated by plotting the reflectance curves of plants selected according to their external appearance. Fig. 8 shows reflectance spectra from a previous EP experiment (data unpublished). A gradual decrease in green and NIR, and an increase in red is seen as symptoms of stress within a treatment increase. At their final stage, when plants are completely senescent the curve shows very little resemblance to that of a typical plant spectrum, lacking the characteristic Chl and water absorption features. Although petroleum exudates were cleaned from stems before the measurements, the possibility of the influence of petroleum itself on the observed changes in reflectance cannot be eliminated.

Table 4
Effect of 'Escravos' petroleum concentration on plant biometric and physiological parameters

Parameter	Treatment			Significance
	C	L	H	
Fv/Fm (last date)	0.84	0.84	0.78	ns
Height (mm)	300.5 a	268.0 b	201.2 c	0.01
Stem thickness (mm)	2.73 a	2.18 b	2.18 b	0.01
Shoot dry weight (g)	2.63 a	1.60 b	1.02 b	0.01
Shoot water content (%)	85.80 a	75.41 b	67.61 c	0.01
Total pigment ($\mu g\ cm^{-2}$)	10.73	9.62	9.05	ns
CO_2 assimilation ($\mu mol\ CO_2\ min^{-1}\ cm^{-1}$)	3.20 a	0.92 b	0.59 b	0.01

Treatment levels: C = control, L = low, H = high. Significance: ns = non-significant at the 0.05 level. Means with different letters are significantly different at the 0.05 level.

Petroleum may cause plant stress due to the toxicity of some of its components, such as aromatic compounds (Suprayogi and Murray, 1999). Oil can also decrease root aeration (Pezeshki et al., 2000) and change the nutrient solubility and uptake. Since hydrocarbons can be

Table 5
Effect of 'Alba' petroleum concentration on plant biometric and physiological parameters

Parameter	Treatment			Significance
	C	L	H	
Fv/Fm (last date)	0.80	0.78	0.80	ns
Height (mm)	583.1 a	402.3 b	377.8 b	0.01
Stem thickness (mm)	2.77	2.75	2.66	ns
Shoot dry weight (g)	53.49	37.41	16.61	ns
Shoot water content (%)	82.39 a	75.72 b	77.77 ab	0.05
Total pigment ($\mu g\ cm^{-2}$)	4.37 a	4.03 a	2.54 b	0.01
CO_2 assimilation ($\mu mol\ CO_2\ min^{-1}\ cm^{-1}$)	5.13 a	3.29 ab	2.48 b	0.05

Treatment levels: C = control, L = low, H = high. Significance: ns = non-significant at the 0.05 level. Means with different letters are significantly different at the 0.05 level.

absorbed by roots and accumulated in stems and leaves (Baker, 1970) it is possible that the most important effect of EP on *Salicornia* was a gradual deterioration of the growing conditions that slowly impaired the normal functioning of the plant (Baker, 1970), and not necessarily a drastic shoot damage that would have had a direct effect on leaf pigments or stem tissue structure.

Light weight petroleum types are known to be more harmful than heavy types (Pezeshki et al., 2000). Heavy petroleum has a higher proportion of higher molecular weight compounds, which are difficult to transport and distribute within the plant. It is also possible that light weight compounds, more abundant in lighter petroleum, are more toxic to plants. It has also been suggested that lighter oils are more toxic than heavy oils in oil spills in wetlands because light oil is less viscous and infiltrates more effectively into the soil and root zone (IPIECA, 1994).

Salicornia proved to be sensitive to the Cd concentrations applied in this study. Because of the non-linear response of plants to increasing levels of Cd it is difficult to clearly establish a lowest effect concentration. Whatever the reason for the lack of linearity, our results indicate that even the lowest concentration (10 ppm) given the right length of exposure would cause measurable effects in most of the parameters considered, including reflectance.

Salicornia also showed sensitivity to petroleum contamination, most notably, to light petroleum. EP produced significant decreases in growth and photosynthesis even at 7 mg g^{-1} of soil after 10 days of exposure, while 14 mg g^{-1} was sufficient to produce significant changes in reflectance. These results agree with studies on the effect of oil on *Spartina alterniflora* at different dosages in soils (Alexander and Webb, 1987), but in contrast with similar studies on the same species in

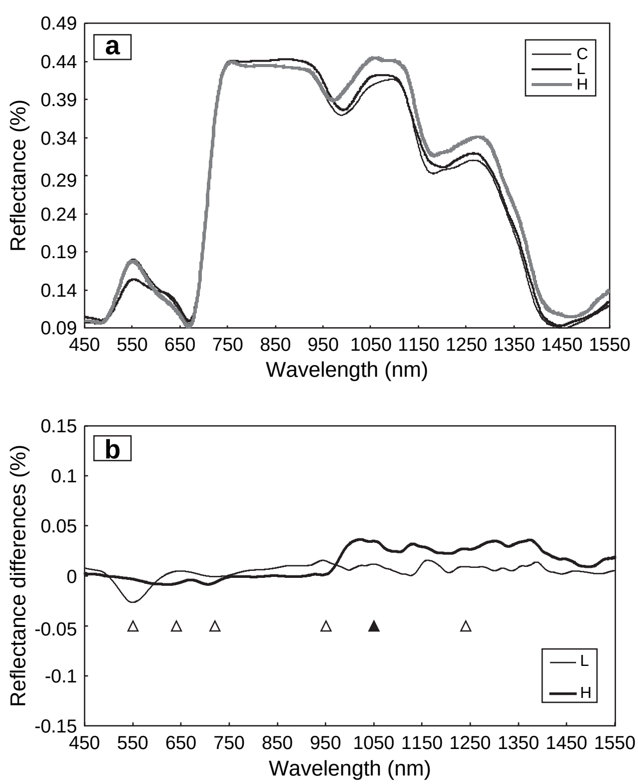

Fig. 7. (a) Reflectance spectra of 'Alba' petroleum experiments, (b) differences between treatment and control spectra. Triangles indicate wavelengths at which differences were tested for significance. Black triangles indicate significant differences at the 0.05 level and white triangles, non-significant differences. C = control, L = low, H = high.

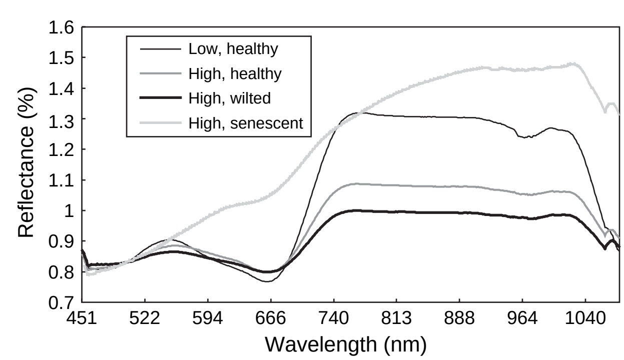

Fig. 8. Reflectance spectra of selected *Salicornia* plants subject to petroleum contamination. Low and High indicate the contamination level. Healthy, wilted and senescent refer to their relative appearance within treatments.

which more than 228 $mg\,g^{-1}$ was necessary to produce significant decreases in plant biomass (Lin et al., 2002). Significant reduction in photosynthesis rate and growth was found at 8 $L\,m^{-2}$, the lowest concentration of a light petroleum tested (Pezeshki et al., 2000). Concentration levels in our study, expressed in $L\,m^{-2}$, ranged from 0.3 to 0.6 (EP) and from 2.31 to 2.73 (AP). However, comparisons with previous studies should be done carefully due to obvious differences in the way the oil was applied, in species and even seasonality of the experiment.

5. Conclusions

Our results suggest that reflectance can be effectively used to detect plant stress due to pollution. The tight relationship between pigment concentration and reflectance in the Cd experiment suggests that reflectance has a great potential for stress prediction. Spectral properties can also be useful in discriminating stress caused by pollutants with different ways of action such as heavy metals and petroleum. The extent to which our findings at the leaf level can be extrapolated to canopy level reflectance should be investigated next.

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